Karolinska Institutet

Institution för Mikrobiologi, Tumör och cell Biologi

Role of angiogenesis in cancer invasion and metastasis

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Petrén, Nobels väg 12b, Solna

Fredagen den 6 december 2013, kl 13.00

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Stockholm 2013

ABSTRACT

Cancer metastasis is a complex process that involves an elaborate interplay between malignant cells and various host cellular and molecular components. The metastatic cascade consists of several discrete but non-separable steps, including: 1) malignant transformation of cells; 2) intravasation and dissemination of tumor cells into the circulation or lymphatic system; 3) transport of malignant cells to distal organs and tissues; 4) adherence of tumor cells to distal sites; 5) formation of the initial metastatic niches, and 6) angiogenesis-dependent re-growth of metastatic foci to clinically detectable tumors. Despite technological advances, advanced image analyses only allow detection of a relatively large tumor-mass in human cancer patients and in various animal models.

Hypoxia is one of the characteristic features of the tumor microenvironment and low oxygen is known to induce tumor angiogenesis. However, the causal link between tumor hypoxia and tumor invasion remains elusive. In this thesis work, we have developed a novel zebrafish model that allows us to investigate tumor invasion at the single cell level in living animals. Furthermore, exposure of the zebrafish in hypoxic water permits us to study the role of hypoxia in facilitating tumor invasion at the early stage of the metastatic cascade. We further investigated hypoxia-regulated genes in tumors that contribute to metastasis. In paper I, we developed a novel zebrafish model to study metastasis at the single cell level in living animals. We take advantage of the transparent nature of zebrafish embryos that are immune privileged at the early stage of development. Additionally, the availability of a transgenic zebrafish line that expresses enhanced green fluorescent protein allows us to study the interaction between tumor cells and the vasculature in a non-invasive manner. We also developed a novel hypoxia chamber in which oxygen levels in the aquarium can be adjusted to a certain desired level. Development of these novel methods allows us to study the early events of cancer metastasis, which otherwise cannot be visualized in mammalian systems. In paper II, we studied the role of hypoxia in promoting metastasis using the method described in Paper I. We have found that hypoxia induces angiogenesis in the implanted tumors and VEGF is the crucial mediator that is responsible for hypoxia-induced tumor angiogenesis. To further validate the role of VEGF in mediating tumor invasion and metastasis, blocking VEGFR signaling by tyrosine kinase inhibitors or specific morpholinos inhibits hypoxia-induced metastasis. Moreover, overexpression of VEGF in tumor cells also significantly promotes cancer metastasis through stimulation of tumor angiogenesis although VEGF lacks direct effects on tumor cells. These findings show that hypoxia plays a pivotal role in facilitating tumor cell dissemination at the early stage of the metastatic cascade and inhibition of the VEGF signaling might be an important approach for treatment of metastatic disease. In paper III, we show that a novel mechanism of hypoxia-induced angiogenesis, which involves in physical interaction between filamin A and HIF-1α. Hypoxia-induced cleavage of filamin A promotes nuclear accumulation of HIF-1α, leading to up-regulation of its target genes such as VEGF. Via this mechanism, filamin A stimulates tumor angiogenesis and tumor growth. In paper IV, we show that filamin B serves as a negative regulator for tumor invasion and metastasis. In filamin B deficient mice, increased metalloproteinase-9 (MMP-9) activity has been detected. Similarly, silencing of MMP-9 in various tumor models resulted in enhanced tumor angiogenesis and invasion by increasing the bioavailability of VEGF.

ISBN 978-91-7549-392-3